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Research Article

ASSESSMENT OF THE HEPATOPROTECTIVE POTENTIAL OF ETHANOLIC EXTRACT OF *LUFFA AEGYPTIACA* MILL. IN ALCOHOL-INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

Oxidative damage is implicated in the pathogenesis of various liver injuries. The study was aimed to investigate the antioxidant activity of fruit of *Luffa aegyptiaca* Mill. on ethanol induced oxidative stress in female wistar rats. The intoxication of rats with 50% ethanol with dose of 4g/kg body weight orally, for a period of 21 days induced oxidative stress, indicated by a significant rise in serum markers of liver injury, such as alanine aminotransferase, aspartate aminotransferase, bilirubin, and disturbances in lipid profile. Impairment in antioxidant mechanisms which involves catalase, superoxide dismutase, reduced form of glutathione was also observed. Post treatment of rats with different doses of *Luffa aegyptiaca* Mill. Fruit ethanol extract (250 and 500 mg/kg) significantly normalized disturbed parameters. Histopathological studies showed severe liver damage, hepatocellular degeneration/ necrosis, inflammation, and dilatation of sinusoids due to ethanol intoxication. Oral administration of the fruit extract at a dose of 500mg/kg body weight significantly reduced the above-mentioned toxic effects of ethanol. The activity of fruit extract at the dose of 500 mg/kg was comparable to the standard drug, silymarin. Based on these results, it was observed that *Luffa aegyptiaca* Mill. fruit extract exhibits potent antioxidant activity and protects liver from oxidative stress induced by ethanol. This helps in evaluation of traditional claim on this plant in the treatment of liver ailments.

Keywords: *Luffa aegyptiaca*; Antioxidant; Ethanol; Hepatoprotective Activity

INTRODUCTION

Liver toxicity a most frequent adverse event reported in Pharmacovigilance safety reports and important reason for the withdrawal of approved medicinal agents from the market as per the data provided by European Medicines Agency (EMEA). Alcohol abuse is one of the major health problems worldwide and a close relationship exists between it and alcoholic liver disease (ALD) (1). In addition, as ethanol is used as one of the excipients in most of the syrups, tinctures, and medicines, one cannot avoid their exposure to ethanol intake though having knowledge of its toxic effects (2). Ethanol induced hepatotoxicity is associated with mechanisms such as free radical generation, lipid peroxidation, antioxidant deficiency, and oxidative stress (3,4). Botanicals that are used in the treatment of liver diseases with a pledge of pharmacological evidence are often inadequate. Hence there is a need to screen some alternative herbs for the treatment of liver diseases which can either replace the currently used drugs of doubtful efficacy and/or safety or at least have a supplementary effect to existing one. *Luffa aegyptiaca* Mill. (Cucurbitaceae) (Synonym- *Luffa cylindrica*) is a climber with slender, slightly hairy, furrowed stem commonly known as sponge gourds (5). Fruit tincture is used in the treatment of jaundice, biliary and intestinal colitis and other disorders related to spleen (6). Ethanolic extract of *Luffa aegyptiaca* fruits (EELA) showed antioxidant activity (7) and hepatoprotective potential against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats in our previous work (8). The objective of this study was to check the hepatoprotective efficacy of *Luffa aegyptiaca* Mill. fruits in ethanol-induced hepatotoxicity in order to produce more evidences regarding its hepatoprotective potential, as well as to check existence of relation between antioxidant and hepatoprotective potential of extract.

MATERIAL AND METHODS

Chemicals and Drugs: Absolute ethanol, other solvents, and chemicals used were of analytical grade and purchased from SD fine chemicals, Mumbai, Maharashtra, India. silymarin was a gift sample from Micro labs, Bangalore, Karnataka, India. Kits for biochemical parameters such as Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), total protein, total bilirubin and direct bilirubin, triglycerides (TGs), serum urea were purchased from Coral clinical systems, Goa, India.

Plant Material: Fresh fruits of *Luffa aegyptiaca* Mill. were purchased in the month of August from a local market in Mumbai, India and authenticated by Dr. Ganesh Iyer, H.O.D. Department of life science, Ramnarain Ruia College, Matunga, Mumbai, India.

Extraction: The fruits were collected, thoroughly washed, sliced and shade dried at room temperature for four to five days. Dried slices of fruits were powdered and 70g was then extracted with ethanol (50%) as a solvent by using a Soxhlet apparatus at 50°C. After extracting all coloring matter, the extract was concentrated under reduced pressure in the rotary evaporator to get brownish semisolid extract. (7,8).

Phytochemical Analysis: Phytochemical screening of fruit extract was carried out to check the presence of carbohydrates,

proteins, alkaloids, glycosides, saponins, steroids, terpenoids, flavonoids, tannins, fats and oils qualitatively (9).

Animals: The experimental protocol designed for the present study was approved by the Institutional Animal Ethics Committee (IAEC) of APT Testing & Research Pvt. Ltd (formerly known as the National Toxicology Centre, NTC), Pune, India, (Regt. No. 40/CPCSEA/1999). Healthy adult female Wistar rats (150-180 g) and female Swiss albino mice (20-25 g) were purchased from APT testing and Research Pvt. Ltd and used for the study. They were housed in small polypropylene cages (32.5 × 21 × 14) cm and (24 × 14 × 12) cm respectively, lined with raw husk. Animals were maintained under controlled environmental conditions (25 ± 2°C and 30-70 % humidity, 12 h light and dark cycle) with free access to food and water. Animals were supplied with the standard chow during the period of study. All experiments were carried out following the guidelines that are given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate change, Government of India.

Toxicity Study: An acute oral toxicity study was performed as per OECD guideline 425 (10).

Hepatoprotective Study (Curative Activity): Curative model for hepatoprotective activity was carried out as described by Pramyothin et al. (11). Normal, healthy female Wistar rats were divided into five groups (n=6). The feeding scheme was as follows:

Group I- Vehicle Control rats received 5 g/kg of distilled water orally for 28 days.

Group II- Disease control rats received 4 g/kg of ethanol (10.85 M) orally for 21 days and 5 g/kg of water once a day orally from day 22 to day 28.

Group III- These rats received 4 g/kg of ethanol (10.85 M) orally for 21 days and 250 mg/kg of EELA once a day orally from day 22 to day 28.

Group IV- These rats received 4 g/kg of ethanol (10.85 M) orally for 21 days and 500 mg/kg of EELA once a day orally from day 22 to day 28.

Group V-Standard control rats received 4 g/kg of ethanol (10.85 M) orally for 21 days and 5 mg/kg of silymarin once a day orally from day 22 to day 28.

On the 29th day, blood was collected by cardiac puncture under urethane anesthesia and animals were sacrificed. The blood samples were kept at room temperature for one h for clotting followed by centrifugation at 2500 RPM at 37°C for 10 minutes to obtain serum and used for estimation of serum parameters such as ALT, AST, ALP, total protein, total bilirubin and direct bilirubin, TGs and serum urea using diagnostic kits (Coral clinical systems, Goa, India) and auto analyzer (Screen master- 3000). Immediately after sacrificing the animals, liver of animals was separated, washed with buffer (pH 7.4), blotted with filter paper. Liver tissue was divided into two portions. A first portion was minced and then homogenized in buffer (pH 7.4) to prepare 10% w/v tissue homogenate. This homogenate was centrifuged at 3000 RPM at 4°C for 15 min. The resulting supernatant was used for estimation of lipid peroxidation, which is expressed as malondialdehyde (MDA) level (12), reduced glutathione (GSH) content (13), catalase (CAT) (14) and superoxide dismutase (SOD) (15).

The second portion of liver was fixed in 10% v/v formalin solution and serially sectioned and microscopically examined by using hematoxylin and eosin (100X) as staining agents.

Statistical Analysis

Results are expressed as mean ± SEM where n=6 (Number of animals per group). Total variation present in the set of data from each group was estimated by one-way analysis of variance (ANOVA) followed by Dunnett's test. A p-value <0.05 was considered statistically significant when compared with disease control group. Indications are as follow $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

RESULTS

The Phytochemical Screening: The final yield of the extract was found to be 28.5 % of the dried weight of the fruits. The results of the preliminary phytochemical screening of EELA showed presence of carbohydrates, proteins, glycosides, saponins, flavonoids, steroids and terpenoids.

Acute Oral Toxicity Test: The EELA did not produce any mortality when administered orally up to dose of 2000 mg/kg. There were no signs of delayed toxicity and mortality also when animals were monitored for a further 14 days. Thus, two doses of extract, i.e. 250 mg/kg and 500 mg/kg were selected for the further study.

Effect of Various Treatments on Serum Parameters: Effect of ethanol and post treatment with EELA/silymarin on various serum parameters are shown in table 1. Administration of ethanol toxin in rats shows significant elevation of serum ALT, AST and ALP. Treatment with EELA significantly inhibited ethanol-induced elevation in all the three markers of liver injury in a dose-dependent manner. The dose of 250 mg/kg was sufficient to significantly show this effect (P value <0.05). Proteins level was drastically reduced due to ethanol intoxication. Treatment with EELA returned protein level to normal, where a dose of 500 mg/kg shown a most significant effect (P value <0.01). Intoxication with ethanol caused a rise in both total and direct bilirubin. EELA was able to reduce the level of total and direct bilirubin. However, the effect on the total bilirubin level was most significant at a dose of 500 mg/kg while a dose of 250 mg/kg was sufficient to significantly reduce the direct bilirubin level in serum. Ethanol administration showed a rise in serum level of urea. However, the effect of EELA on ethanol induced rise in serum urea was not significant.

Effect of Various Treatments on Liver Antioxidant Status: It was observed in figure 1-4 that, administration of ethanol toxin in rat causes significant elevation of tissue injury parameter such as MDA and depletion in antioxidants which includes components like GSH and enzymes such as CAT and SOD. Treatment with EELA significantly cured ethanol induced pathological changes in dose-dependent manner.

Histopathological Observations: Rats from all the groups were studied for cellular architecture of the liver tissue by histopathological analysis and micrographs are shown in figure 5-9.

Table 1: Effect of various treatments on serum biochemical parameters

Parameters	Vehicle control	Disease Control	Extract dose (250 mg/kg)	Extract dose (500 mg/kg)	Silymarin (5mg/kg)
ALT (IU/L)	49.667±2.848***	95.333±2.275	78.167±0.910*	61.333±4.447**	55.500±2.078***
AST (IU/L)	169.667±4.856***	324.833±6.483	267.333±4.595*	212.333±8.164**	188.667±6.173**
ALP (IU/L)	235.833±1.886***	604.000±1.832	459.667±5.524*	349.167±2.680**	296.833±0.141***
Total proteins(g/dl)	7.8±0.253***	4.283±0.268	5.4±0.271	7.133±0.302**	7.583±0.218***
Total bilirubin (mg/dl)	0.102±0.013***	0.540±0.016	0.428±0.018	0.235±0.029**	0.197±0.014**
Direct bilirubin (mg/dl)	0.050±0.012***	0.310±0.018	0.207±0.012**	0.125±0.023***	0.10±0.013***
TGs (mg/dl)	52.667±2.155***	108.500±3.284	86.00±2.543*	68.00±4.830**	61.833±2.330**
Serum Urea(mg/dl)	27.167±0.872**	36.167±1.195	34.0±0.632	32.33±0.760	32.0±1.065

Results are expressed as mean ± SEM where n=6. Statistical evaluation done by one-way analysis of variance (ANOVA) followed by dunnett's test. p-value <0.05 was considered statistically significant when compared with disease control group. Indications are as follow *p<0.05, **p<0.01, ***p< 0.001

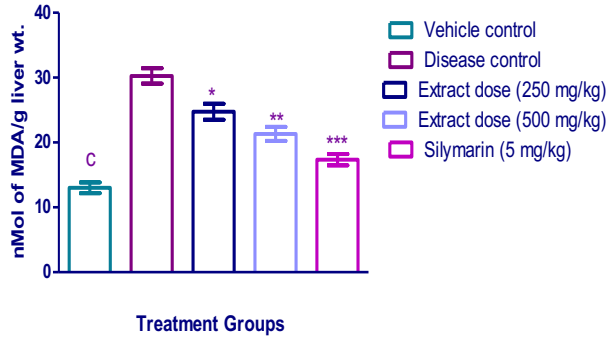

Figure 1: Effect of various treatments on MDA level

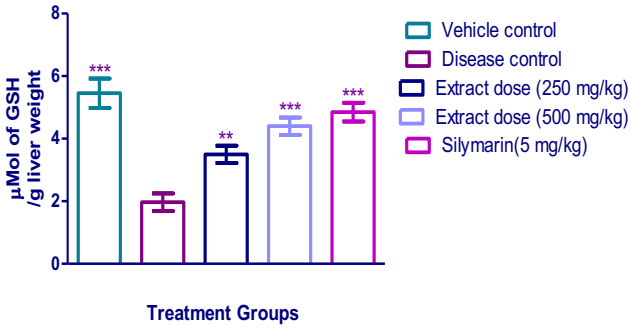

Figure 2: Effect of various treatments on GSH level (H&E x 100X)

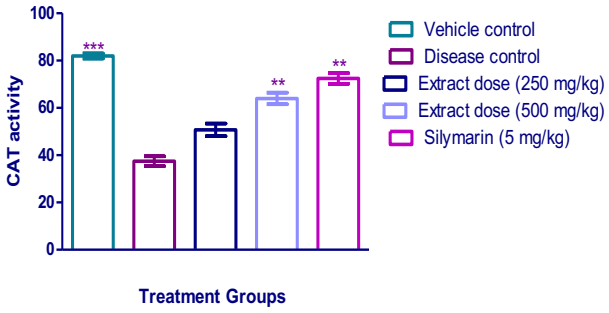

Figure 3: Effect of various treatments on CAT activity

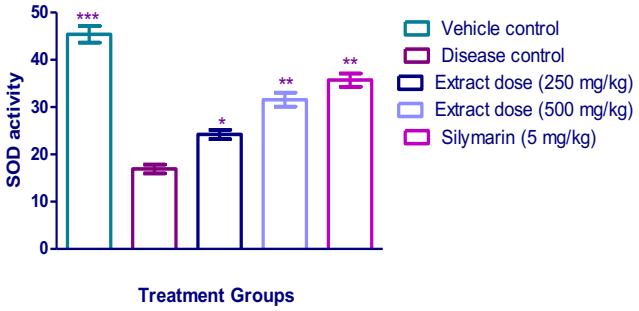

Figure 4: Effect of various treatments on SOD activity

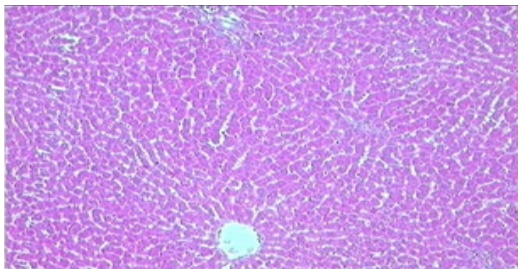

Figure 5: Group I Vehicle Control: Normal architecture of hepatic cells and no abnormalities (H&E x 100X)

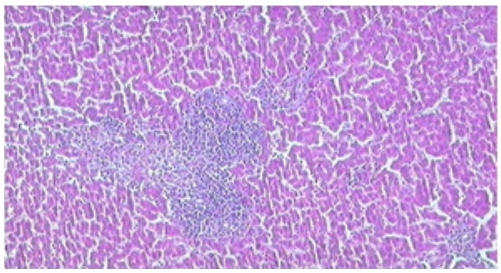

Figure 6: Group II Disease Control: Distorted architecture with extensive area of necrosis, marked sinusoidal congestion with focal lobular mononuclear cell infiltrate (H&E x 100X)

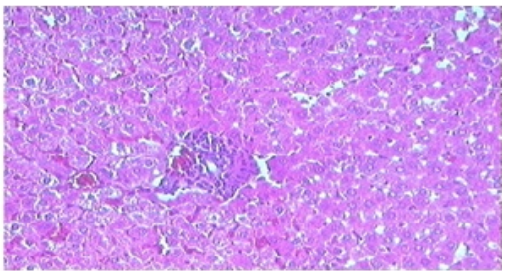

Figure 7: Group III Extract dose (250 mg/kg): Lobular and periportal mononuclear cell infiltrates and sinusoids contain few mononuclear cells (H&E x 100X)

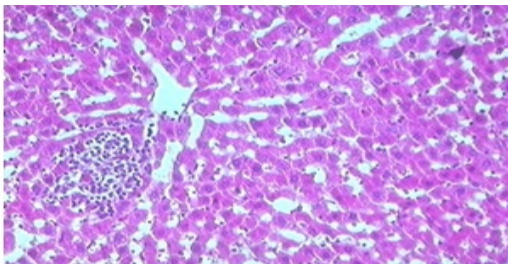

Figure 8: Group IV Extract dose (500 mg/kg): Scanty focal lobular and mononuclear cell infiltrate; otherwise no abnormalities (H&E x 100X)

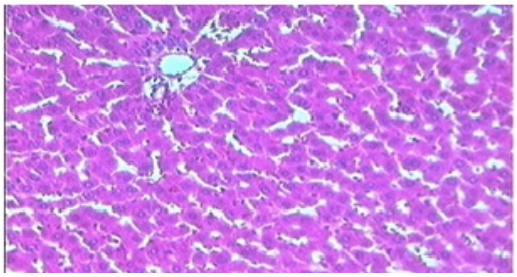

Figure 9: Group V Silymarin (5mg/kg): Minimal inflammation and no significant abnormalities (H&E x 100X)

DISCUSSION

EELA revealed the presence of more than one phytochemical. From several decades alkaloids, flavonoids, glycosides, saponins are known to possess hepatoprotective activity in animals. So in the present state of knowledge of the chemical constituents of the EELA, it is not possible to attribute with certainty the hepatoprotective effects to one active phytoconstituent. In liver injury, the transport function of the hepatocyte is disturbed, resulting in the leakage of cell membrane, thereby causing an increased in some enzyme level in serum. The elevated activities of AST, ALT and ALP in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in the liver. Similar observations were made on administration of ethanol in this study. Oral administration of EELA at a dose of 250 mg/kg and 500 mg/kg to rats caused a decrease in the activity of the above enzymes, suggesting that EELA may cause the plasma membrane stabilization and also involved in repair of hepatic tissue damage caused by ethanol. Ethanol is known to cause injury in hepatic parenchymal cells, which causes elevation in serum bilirubin levels. Hyperbilirubinaemia is a very precise test to detect the functional integrity of the liver and severity of necrosis. Reduction in bilirubin level along with the suppression of the activity of ALP in the serum of rats in Group III and IV suggests the possibility of the EELA being able to stabilize biliary dysfunction of rats caused by ethanol. In hepatotoxicity, protein synthesis is reduced due to the disruption in the process of mRNA assembly with the ribosome and translation following ethanol administration. EELA prevented this change suggest that it possibly promotes the assembly of ribosome's on endoplasmic reticulum to facilitate protein biosynthesis without any interruption. Increased level of Acetyl CoA upon ethanol metabolism, inevitably increase plasma low density lipoproteins (LDL) levels and disturbs lipid profile in rats. The remodeling of ethanol provoked changes in the serum lipid profile shows the protective nature of EELA. Urea is the end product of protein metabolism. Administration of ethanol alters kidney functions and induces oxidative stress mediated rise in serum urea level. However, both extract and standard drug failed to bring changes in urea level to normal value significantly. This may be due to lack of efficacy in preserving kidney functions.

MDA is a major reactive aldehyde, a product of peroxidation of biological membrane polyunsaturated fatty acid (PUFA). Rise in MDA level is an indication of tissue damage due to lipid peroxidation of cell membrane. It has been hypothesized that free radical induced lipid peroxidation in the cell membrane is one of the important causes behind ethanol induced hepatotoxicity. The observed values of elevated levels of hepatic MDA in Group II rats in the present study are consistent with this hypothesis. Thus, the maintenance of hepatic MDA to near normal level in Group IV rats is of great interest. Glutathione is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant presents in liver. Its functions are concerned with the removal of free radicals such as superoxide radicals, maintenance of membrane protein, biotransformation of drugs and detoxification process. GSH, which is a reduced form of glutathione, plays a key role in the detoxification of the reactive toxic metabolites. Its supplement protects the liver from necrosis, which is generally seen when reserves of GSH are markedly depleted. The reduced levels of GSH observed in the present investigation in group II rats may occur due to increase in its efflux from the liver, inhibition of its biosynthesis and complex formation with acetaldehyde or due to increased utilization of GSH by enzymes such as glutathione peroxidase. Interestingly, in the present study, Group III and IV rats had a mean GSH level that was quite higher than that in Group II rats and close to the value obtained in Group I. The EELA may act through induction of the glutathione biosynthesis or induction of other detoxifying enzymes and these enzymes may detoxify the reactive oxygen species (ROS) produced due to ethanol. CAT and SOD are the most sensitive enzymatic indices in liver injury caused by ROS and oxidative stress. CAT is a haemoprotein; it protects the cells from the accumulation of H_2O_2 by acting upon it to form H_2O and O_2. Whereas SOD is intracellular antioxidant enzymes present in all aerobic cells and it has an antioxidant effect against ROS. A reduction in the activities of these enzymes ultimately results in the accumulation of highly reactive free radicals. This ends with loss of integrity and function of cell membranes. Administration of ethanol is associated with inactivation of CAT and SOD via free radicals. This probably explains the reduced activities of CAT and SOD observed by us in rats challenged with ethanol. In rats receiving ethanol and EELA, the activities of CAT and SOD is significantly higher and very similar to the values observed in normal rats. The results from histopathological studies also provided supportive evidences.

CONCLUSION

The impact of EELA on various biochemical and tissue injury markers suggests that EELA can reduce ROS, oxidative damage, improve the activities of the liver antioxidant enzymes and supports our consideration about the hepatoprotective role of EELA is due to its antioxidant potential.

However, the principal components responsible for this activity and the mechanism of action at the molecular level are currently unclear. Further isolation of phytochemicals and their characterization using modern analytical technologies are required to identify the active principles responsible for the antioxidant and hepatoprotective activity. There is urging to search for the existence of any synergism, if any, among the phytochemical present in EELA, in future. Furthermore, to confirm the mechanism of action on liver organelles that are possibly damaged during experimental hepatotoxicity and to develop a potent hepatoprotective agent with low toxicity and better therapeutic index, extensive studies at the cellular and molecular level should be carried out. Thus, from this study we

have proved the efficacy of *Luffa aegyptiaca* Mill. fruit. in ethanol induced hepatotoxicity and once again confirm the traditional claim for its hepatoprotective potential.

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